Altitudinal genetic variation among *Pinus oocarpa* populations in Michoacán, Mexico Implications for seed zoning, conservation, tree breeding and global warming

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Abstract

Pinus oocarpa has a large natural distribution in the sub-tropical forests of Mexico. Populations, however, are rapidly disappearing particularly in the Michoacán State as native forests are converted to avocado (*Persea* sp.) orchards. We investigated the patterning of genetic variation among *P. oocarpa* populations for quantitative traits along an altitudinal gradient by establishing a provenance/progeny test from wind-pollinated seeds collected along an altitudinal transect (1100–1500 m) near Uruapan, Michoacán, Mexico. Genetic variation was analyzed in relation to normalized climate records (temperature, precipitation, degree days $>5\,^{\circ}C$ and annual moisture index) for the provenances and the test site for the contemporary climate and for climates projected for the decades beginning in 2030, 2060, and 2090. Estimates of future climates used output from the Canadian and Hadley General Circulation Models.

Results of a field test suggested an altitudinal pattern of genetic differentiation in juvenile height among *P. oocarpa* populations. Seedlings from populations originating from lower altitudes tended to grow more than seedlings originating from populations at the higher altitudes. However, this trend abates at the lowest altitudinal limit of the species distribution, a probable conservative growth strategy for avoiding drought stress. Thus, the cline appeared to arise from selection along a climatic gradient reflecting in a moisture index (ratio of degree days to precipitation) and is dependent, therefore, on a balance between temperature and moisture. For guiding seed and seedling transfer in ecological restoration, conservation of genetic resources, tree breeding and mitigating the effects of global warming, we suggest guidelines based on delimitation of three altitudinal seed zones of about 200 m in breadth. Alternatively, one can limit transfer to three climatic zones of about 0.75 units of annual moisture index.

Predictions of future climates indicate an average annual temperature increase of $3.8\,^{\circ}C$ by year 2090, and, judging from an increase of an annual moisture index of 26%, an increase in aridity by the end of the century. However, the more difficult period for adaptation of *P. oocarpa* populations to the new climate should be between 2030 and 2060, when the increase in aridity is expected to be most pronounced. Changes of this magnitude should alter the natural distribution of the species and would create an adaptational lag, as the adaptedness of extant populations deteriorates. Mitigating these effects will require seeds to be transferred upwards in altitude, perhaps as much as 150 m initially.
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1. Introduction

Conifer populations tend to be differentiated genetically along altitudinal gradients in response to natural selection. In

general, populations from mild environments at low altitudes have larger growth potential, longer period of shoot elongation, and less resistance to frost damage, than populations that originate from colder environments at higher altitudes. High altitude sources show, therefore, lesser growth potential, shorter periods of shoot elongation and more resistance to frost damage (Campbell, 1979; Rehfeldt, 1988, 1989, 1991). Such clinal patterns have not been documented for populations of Mexican pine species, but their occurrence is likely simply because they share a similar life history and montane distribution in the Sierra Madre Oriental, Sierra Madre Occidental, and Neovolcanic Axis (see Perry, 1991) as those conifers for which clines are well documented.

Among the numerous pines species that occur in Mexico, *Pinus oocarpa* has the largest north–south natural distribution (Perry, 1991; Dvorak et al., 2000). This species is the country's most important pine for resin production (COFOM, 2001), and is also used for firewood, saw timber and plywood (Zamora-Serrano, 1981). In addition, extensive commercial plantations of this species are grown as exotics in South Africa and South America (Greaves, 1982; Dvorak et al., 2000).

Despite the ecological and economic importance of *P. oocarpa* to Mexico, natural populations are rapidly disappearing due to illegal logging, forest fires and deforestation associated with conversion of lands for agriculture, fruticulture and pasture (Farjon and Styles, 1997; Dvorak et al., 2000; COFOM, 2001; Sáenz-Romero and Tapia-Olivares, 2003). In the western Mexico State of Michoacán, deforestation amounts approximately 35,000 ha/yr (COFOM, 2001). Although the proportion of these hectares occupied by natural populations of *P. oocarpa* is not known, deforestation of this species in the region of Uruapan (120 km west of Morelia, the State Capital of Michoacán) is extensive. Natural forests are being replaced by

avocado (*Persea gratissima* and *Persea americana*) orchards (Sáenz-Romero and Tapia-Olivares, 2003). *P. oocarpa* has the lowest altitudinal range (1100–1600 m) among several pine species that naturally occur in this region and occupies, therefore, a climatic niche that overlaps with avocado. Mexico now leads the world in avocado production (34% of the worldwide production), and Michoacán leads Mexico (85% of the national avocado production, AALPAUM, 2005). Consequently, there is and has been a strong economic incentive for converting *P. oocarpa* forests to avocado orchards.

Climatic conditions of the Uruapan region approach the median of the climatic niche of *P. oocarpa*. Populations at Uruapan region are: (a) situated near of the geographic center of the north-west to south-east axis of the species' geographic distribution (Fig. 1), (b) dispersed across an elevational range (1100–1600 m) that is approximately intermediate for the species' altitudinal extremes (lowest at 200 m and highest at 2500 m, Perry, 1991), and (c) exposed to an average annual precipitation range (1313–1640 mm, this study) that is intermediate between the dry-temperate conditions of the north-west extreme limits of distribution (500–1000 mm at the Mexican states of Sonora, Chihuahua and Sinaloa) and the humid-tropical conditions at the south-east limits (2000–3000 mm in Belize, Honduras and Nicaragua, Perry, 1991).

Because of its large natural distribution, *P. oocarpa* cannot be considered an endangered species. Yet, removal of locally adapted populations implies loss of a valuable forest genetic resource (Dvorak et al., 2000) not only for Mexico but also for all countries growing *P. oocarpa* as an exotic in commercial plantations. Establishing a forest genetic resource conservation program for protecting the remaining within-species genetic diversity first and foremost requires information on the patterns of genetic variation among and within populations
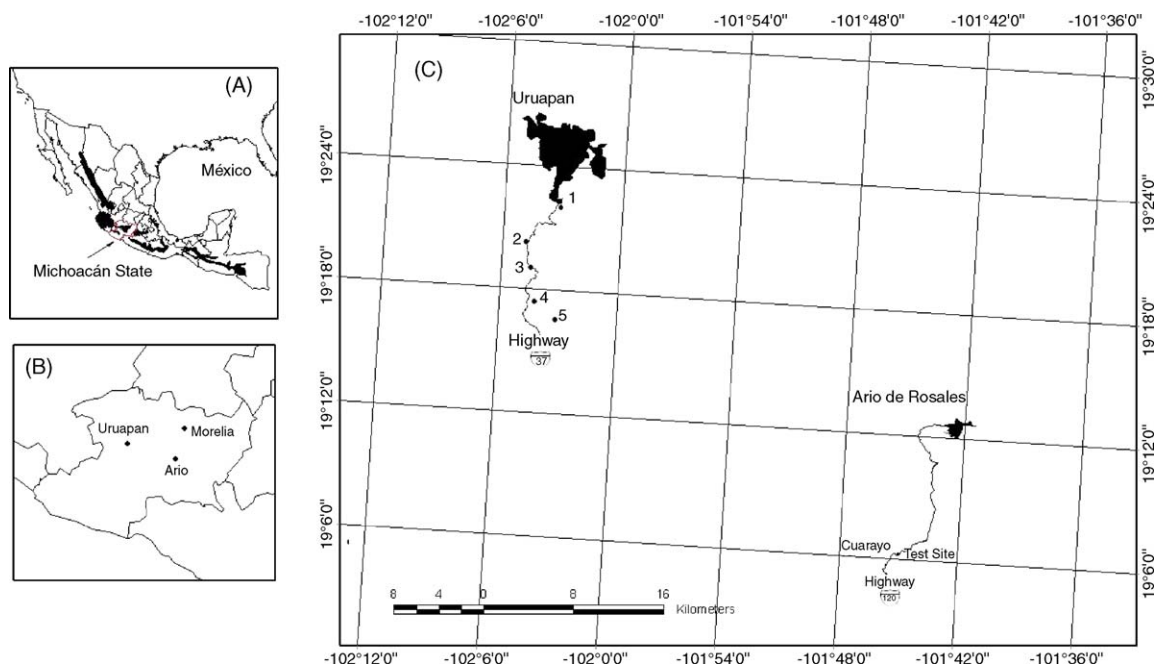

Fig. 1. (A) Natural distribution of *P. oocarpa*, based on Perry (1991). (B) Michoacán state, Mexico. (C) Location of five *Pinus oocarpa* populations (Number codes keyed to Table 1) sampled along an altitudinal transect in the region of Uruapan, and location of the test site, in the region of Ario de Rosales.

(Sáenz-Romero et al., 2003). This information is basic to deciding, for example, the size and location of Forest Genetic Resources Conservation Units (Sáenz-Romero et al., 2003), also called forest genetic resource management units *sensu* Ledig (1988) and Millar and Libby (1991).

Federal and State Mexican government organizations have established a large reforestation program with several forest species to counteract the negative effects of the ongoing deforestation. Reforestation under this program amounts to approximately 200,000 ha/yr nation wide (CONAFOR, 2005) and 16,000 ha/yr for Michoacán State (COFOM, 2002). Unfortunately, seedling survivorship is low, averaging approximately 36% after 1 year of planting nationwide (Sheinbaum and Masera, 2000), and 38% for Michoacán State (SEMARNAP, 2000). One of several causes of this low survivorship is the poor matching between the climate to which seedling genotypes are adapted (*sensu*, physiologically attuned) and the climate of the planting site (Sáenz-Romero and Lindig-Cisneros, 2004).

The environmental conditions that mold genetic variability and thereby control adaptedness, however, are not static. Modeled predictions of global warming considered in terms of the genecology of forest tree populations suggest that within the coming 50–100 years, conifer populations will become more and more poorly adapted to the sites they inhabit today (for example, see Rehfeldt et al., 1999a,b, 2003, 2004; Tchebakova et al., 2003). Research results suggest that managers may need to establish plantations with genotypes suited for future climates rather than with those suited to contemporary climates. One might, for example, move seeds upwards in altitude in anticipation of the future location of the contemporary climate. However, the guidelines for such programs must be based on climatic clines simply because elevation is merely a surrogate for climate.

This paper examines genetic responses of *P. oocarpa* populations of central Mexico in relation to altitudinal and climatic gradients. The results are applied to: (1) delineating of seed zones and developing seed transfer guidelines required for matching genotypes with the appropriate planting site, (2) establishing Forest Genetic Resources Conservation Units, (3) selection of provenances for tree breeding, and (4) developing reforestation guidelines for mitigating adverse effects of global warming on the growth and survival of future forests. To meet these objectives, we examine the pattern of genetic differentiation for quantitative traits along altitudinal gradients inhabited by *P. oocarpa* populations in Michoacán, Mexico. Results are presented in terms of the contemporary climate as well as the climate projected for the decades beginning in 2030, 2060, and 2090.

2. Materials and methods

Wind-pollinated cones were collected from approximately 11 randomly selected trees from each of 5 *P. oocarpa* natural populations distributed along an altitudinal gradient on a southern slope of the Neovolcanic Axis, near Uruapan city (Table 1 and Fig. 1). Sampled populations were separated by

Table 1
Locations for five *Pinus oocarpa* populations sampled along an altitudinal transect in the region of Uruapan and for the test site, Michoacán State, western Mexico

Provenance	Locality	Municipality	Lat N	Long W	Altitude (m)
1	Uruapan	Uruapan	19°21′7″	102°06′3″	1505
2	Matanguarán	Uruapan	19°20′0″	102°05′8″	1430
3	El Catorce	Uruapan	19°18′9″	102°05′0″	1325
4	La Tinaja	Gabriel Zamora	19°17′2″	102°05′8″	1220
5	Charapendo	Gabriel Zamora	19°16′3″	102°06′9″	1075
Test site	Canalejas	Ario de Rosales	19°04′3″	101°44′1″	1490

an altitudinal interval of approximately 100 m (Table 1). The populations were approximately evenly spaced along the elevational transect with an average distance of approximately 4 km. The trees represented by these samples are termed populations while the location of a population is called the provenance.

Seedlings were grown in 380 cm³ pots at Morelia. When the seedlings were 13 months of age, a subsample averaging six half-sib families for each of the five populations was used to establish a field test at the locality of Canalejas, near of Cuarayo village, *Ejido* San José de Cañas, Municipality of Ario de Rosario, Michoacán, Mexico (Table 1). The site of the field test was within the natural distribution of *P. oocarpa* and is located about 45 km east of the Charapendo population in Fig. 1. The test had a randomized complete block design with four blocks.

Seedling heights were measured at 2.5 years after seed germination. At that age, seedlings had spent 1 year in the nursery and 1.5 years (including two June–October rainy seasons) at the field test site. Statistical analyses included analysis of variance to test significance among populations using PROC GLM with the RANDOM statement of SAS (SAS, 1988). Ratio of variance component to total variance was estimated using PROC VARCOMP METHOD = REML (SAS, 1988). These analyses used the following statistical model:

$$Y_{ijkl} = \mu + B_i + P_j + F_k(P_j) + B_i \times P_j + B_i \times F_k(P_j) + e_{ijkl} \tag{1}$$

where Y_{ijkl} is the observation on the lth seedling of the kth family of the jth population in the ith block, μ the overall mean, B_i the effect of the ith block, P_j the effect of jth population, $F_k(P_j)$ the effect of the kth family nested in the jth population, $B_i \times P_j$ the interaction of block by population, $B_i \times F_k(P_j)$ the interaction of block by family nested in population, and e_{ijkl} is the error term, $i = 1, \ldots, b, j = 1, \ldots, p, k = 1, \ldots, t$, and $l = 1, \ldots n$, where $b = 4, p = 5, t = 6$ in average, and $n = 3$, which are the number of blocks, populations, families and seedlings-per-plot, respectively. Blocks, populations and families were considered as random effects from which inferences were to be made about the *P. oocarpa* populations in the Uruapan region.

The average relative magnitude of differences among families within populations (r) was estimated by averaging across populations the ratio of the difference between the

maximum and minimum family average to the population average, using the following formula:

$$r_i = \frac{(X_{MAX} - X_{MIN})}{X_{POP}} \tag{2}$$

$$r = \frac{\sum r_i}{t} \tag{3}$$

where X_{MAX} is the maximum family average, X_{MIN} the minimum family average, X_{POP} the average of the ith population, r_i the ratio for the ith population, t the number of populations, and r is the average relative magnitude of differences among families within population.

The relationship of genetic variation among populations to the altitude and to the climate of the provenance was assessed with a quadratic model, using PROC REG (SAS, 1988). The model was

$$Y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_{ij} \tag{4}$$

where Y_{ij} is the population mean seedling height, β_0 the intercept, β_1 and β_2 the regression parameters, X_i the altitude (m) of ith population origin, and ε_{ij} is the error. Differentiation along the cline was interpreted relative to the confidence interval about predicted heights ($\alpha = 0.2$). These confidence intervals were used to estimate the altitudinal intervals that must separate populations before one can be reasonably certain of genetic differentiation (see Rehfeldt et al., 1999a).

Assessing genetic variation in relation to climate requires records of monthly average temperature and precipitation from long-term weather stations proximal to the provenances and the test site. The current approach to comparing weather data from stations with a variable period of record involves standardizing the records for the period 1961–1990, using as a basis for standardization those stations with complete sets of monthly observations for 28 years of the 30-year period. These weather stations are termed standard stations and their monthly data are considered monthly normals.

Because the Mexican system for maintaining and updating climate data from most of the weather stations is underfunded and poorly equipped, weather records are infrequently collated. For this study, part of weather records was obtained directly from the persons recording the data. It was fortunate for our study that a weather station exists at Uruapan City (19°25′10″N, 102°03′45″W, elevation 1611 m) which is located only 105 m higher in elevation and 6.4 km from population 1, our population from the highest altitude (Fig. 2). The period of record for this station was inclusive from 1962 to 1999, and although 17 monthly observations were missing for average temperature and 20 for precipitation, we considered the record to be sufficiently complete to qualify the station as a standard station. Monthly normals, therefore, were calculated as the mean of all months for the 30-year period. From this standard station, raw data from other stations could be standardized by summing monthly deviations (whether or not from the normal period) for coincident observations and using the mean of the deviations to normalize the raw values (see Rehfeldt, 2006).
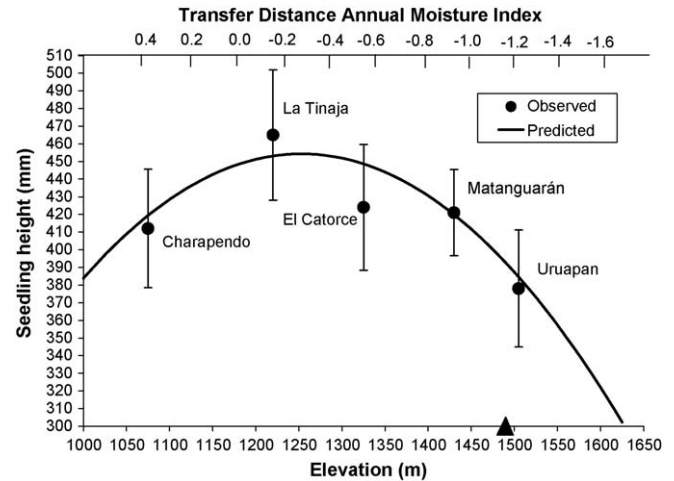

Fig. 2. Population average 2.5-year-old *Pinus oocarpa* seedling height observed and predicted (by a quadratic model) plotted against provenance elevation and against transfer distance in units of annual moisture index from the provenance to the test site. Altitude of Canalejas test site is indicated with a triangle on the elevation *x*-axis. Vertical bars denote 95% confidence intervals.

A second weather station of shorter period of record from 1953 to 1983 was located at El Cobano (19°10′N, 102°01′L, elevation 577 m), 11.2 km southeast from population 5 which was at the lowest altitude in our sample. After normalizing the records for this station, monthly normals of our five *P. oocarpa* populations were interpolated from the altitudinal differences:

$$K_i = \frac{X - Z_i}{X - Y} \tag{5}$$

where K_i is the correction coefficient for estimation of climatic monthly normals for the ith population, X the altitude (m) for nearest upper weather station (Uruapan city), Y the altitude for nearest lower weather station (El Cobano), and Z_i is the altitude for ith population.

Quite similarly, stations located at Ario de Rosales (19°12′50″N, 101°42′0″W, elevation 1910 m), La Sandia (19°15′1″N, 101°40′8″W, elevation 2080 m), and La Huacana (18°58′N, 101°48′W, elevation 550 m) were normalized and used to interpolate the climate at the Canalejas test site. These stations are located approximately 16, 22, and 12 km from the test site. For interpolation, we assumed a direct relationship between altitude and precipitation, an assumption supported by a significant and direct relationship between normalized annual precipitation and elevation of the five weather stations ($r = 0.93$, $p = 0.0215$).

Monthly temperature and precipitation records were converted to five variables of demonstrated importance in plant geography and ecology (see Tuhkanen, 1980): mean annual temperature, mean annual precipitation (mm), summer precipitation (April–September), degree days >5 °C, an annual moisture index (the ratio of degree days >5 °C to annual precipitation), and a summer moisture index (the ratio of degree days >5 °C to summer precipitation) for the population sites and for the test site. Together these variables describe the warmth, moistness, and aridity of the general climate and growing season climate (see Rehfeldt, 2006).

Relationships between provenance climate and seedling performance were assessed with the regression model (4). Independent variables included the four climate variables plus transfer distance in units of climate, calculated as the difference between the climate variable of each of the provenances and that at the Canalejas test site. Note that because climate estimates of the provenances were interpolated from altitude, the relationship between climate transfer distance and altitude of the provenance would be perfect for one test site. This, however, would not necessarily be true for the absolute value of climate transfer distance to a test site of intermediate climate.

To assess the effects of global warming, monthly climate normals of the provenances and the test site were projected for the decades beginning in 2030, 2060 and 2090 by using output of the General Circulation Model (GCM) of the Hadley Centre (Gordon et al., 2000) and the Canadian Centre for Climate Modeling and Analysis (Flato and Boer, 2001) for the IS92a scenario (1% per year increase of greenhouse gases after 1990) of the International Panel on Climate Change (IPCC, 2001). From each GCM, monthly means for average temperature and precipitation were obtained for the normalization period (1961–1990) and for the three future decades. GCM output was used to calculate the monthly change in climate between the normalization period and the future decades for those grid cells that both encompass and surround our provenances and test site. The change in climate expected at each provenance was calculated by weighting the change expected at each cell center according to the distance between the cell's center and the provenance or test site. The inverse of the distance of the provenance to the cell center was used for weighting. Because the two GCM's use different grid sizes, weighted means were calculated for each GCM and were averaged to estimate the future climate of each provenance.

The results are used to develop general recommendations for the transfer of seed and seedlings for maintaining adaptedness in reforestation in the contemporary climate and for mitigating the effects of global warming on adaptedness in the future climate. Application of the results encompasses four managerial objectives: ecological restoration, genetic conservation, tree improvement, and anticipation of global warming effects.

3. Results

Analysis of variance detected significant differences ($p = 0.0368$) among populations, amounting to 3.3% of total variance (Table 2). The largest difference between two populations was between the second altitudinal lowest population ("La Tinaja", 1220 m, average seedling height = 465 mm) and the population originating at the highest altitude ("Uruapan", 1505 m, average seedling height = 378 mm, Fig. 2). The height of the population from La Tinaja was 23% superior to that of the population from Uruapan.

Unexpectedly, there were no significant differences ($p = 0.2312$) among families within populations (Table 2).

Table 2
Analysis of variance for 2.5-year-old seedling height

S.V.	d.f.	Variance component (%)	F^{a}	P
Block	3	5.22	6.42	0.0058
Population	4	3.25	3.64	0.0368
Family (Pop)	25	0.00	1.25	0.2312
Blo × Pop	12	2.68	1.23	0.2781
Blo × Fam (Pop)	61	0.00	0.77	0.8881
Error	190	88.85		

[a] Denominator degrees of freedom (rounded) for F-tests for blocks, population, families and interaction Blo × Fam(Pro) were 14, 12, 68, and 71, respectively.

Average relative magnitude of differences among families within populations was relatively low ($r = 0.2056$), another indication of relatively small differences among family averages within populations.

Mean seedling heights of populations describe a general clinal trend: seedlings from populations originating from lower altitudes tended to grow more than seedlings originating from populations at the higher altitudes. However, this trend abates at the lowest altitudinal limit of the species distribution: the population from the lowest elevation ("Charapendo", 1075 m) grew significantly less than the second lowest population ("La Tinaja", 1220 m) (Fig. 2).

The clinal trend can be described by a quadratic regression model of population average seedling height on provenance elevation ($r^2 = 0.8205$, $p = 0.1795$). Predicted values suggest that the greatest growth at this test site would occur in populations originating from 1255 m of altitude; growth of populations from either lower or higher altitudes should be less (Fig. 2). This hyperbolic response of population growth to climate is well established among conifers (e.g. Rehfeldt et al., 1999b, 2003).

The same clinal trend describes the relationship between seedling growth and provenance climate when the later is expressed as the difference in units of annual moisture index between the provenance and the test site, the "transfer distance". A quadratic regression model of population average seedling height on transfer distance in units of annual moisture index explained approximately 87% of the variance ($r^2 = 0.8682$, $p = 0.1318$, Fig. 2). The fit of this regression model was superior to those of models using transfer distances in other climatic variables: annual temperature, precipitation, degree days and summer moisture index, which yield r^2 and significance p values of $r^2 = 0.8020$, $p = 0.1980$, $r^2 = 0.8199$, $p = 1801$, $r^2 = 0.8203$, $p = 0.1797$, and $r^2 = 0.8282$, $p = 0.1718$, respectively.

The fit of regressions of population height on climate is essentially the same as that on population elevation because climate of the provenances was interpolated from altitude. Consequently, the relation between provenance altitude and provenance climate is perfect. Because there was a single test site, the relationship between provenance altitude and climate transfer distance from the provenance to the test site is also perfect ($r^2 = 0.9961$, $p = 0.0001$). This perfect relationship allows population means to be plotted simultaneously against

Table 3
Present (year 2000) and estimated future (mean for decades beginning in 2030, 2060 and 2090) climate for five *Pinus oocarpa* provenances and the test site

Provenance or site	Locality	Altitude (m)	Year							
			2000	2030	2060	2090	2000	2030	2060	2090
			Temperature (°C)				Precipitation (mm)			
1	Uruapan	1505	20.5	21.8	23.0	24.3	1640	1690	1551	1602
2	Matanguarán	1430	20.9	22.3	23.5	24.7	1583	1632	1496	1547
3	El Catorce	1325	21.6	22.9	24.1	25.4	1503	1550	1419	1471
4	La Tinaja	1220	22.2	23.6	24.8	26.1	1423	1468	1345	1394
5	Charapendo	1075	23.1	24.5	25.7	27.0	1313	1355	1242	1286
Test site	Canalejas	1490	19.7	21.1	22.2	23.5	1164	1209	1100	1151
Provenance or site	Locality	Altitude (m)	Degree days >5 °C				Annual moisture index			
1	Uruapan	1505	5643	6147	6572	7041	3.4	3.6	4.2	4.4
2	Matanguarán	1430	5812	6314	6739	7209	3.7	3.9	4.5	4.7
3	El Catorce	1325	6049	6552	6977	7446	4.0	4.2	4.9	5.1
4	La Tinaja	1220	6282	6792	7217	7686	4.4	4.6	5.4	5.5
5	Charapendo	1075	6613	7120	7545	8014	5.0	5.3	6.1	6.2
Test site	Canalejas	1490	5367	5873	6293	6761	4.6	4.9	5.7	5.9

elevation and against transfer distance in units of annual moisture index simultaneously in Fig. 2.

Although within the natural distribution of *P. oocarpa*, the Canalejas test site tends to be cooler and drier than any of the provenances under test (Table 3). This combination of temperature and precipitation produced a moisture index that was approximately equivalent to the climate that occurs at La Tinaja, although both sites have much different altitudes. Although Canalejas test site is located an altitude (1490 m) similar to the Uruapan provenance (1505 m), Canalejas had the lowest estimated precipitation than any of the provenance sites (1164 mm at present, Table 3). This is reflected on the estimated Canalejas moisture index value: 4.6 at present (Table 3). Such value is close to moisture index value for La Tinaja provenance: 4.4 at present (Table 3), situated at a much lower elevation (1220 m). Note also that the zero value of the transfer distance in units of annual moisture index (*x*-axis from Fig. 2), which means no climatic difference between a provenance and the test

site, corresponds to a point on the curve of predicted values near to that of the La Tinaja provenance. It is consistent with theory on ecologic adaptation, therefore, that the La Tinaja provenance was the best growing provenance at the Canalejas test site (Fig. 2).

Understanding the non-linear response of these populations to the climate at Canalejas is furthered by considering seedling height in relation to the absolute value of the transfer distance in units of the moisture index (Fig. 3). This relationship is essentially linear, with transfer distance explaining 69% of the variance ($r^2 = 0.69$, $p = 0.0806$, Fig. 3) in seedling height. The regression coefficient suggests that provenance transfers of one unit of the annual moisture index should produce a loss of 65 mm of seedling growth in height ($\beta_1 = -65$, Fig. 3). Optimal growth, therefore, involves a transfer to sites with approximately the same moisture index as the provenance. The relationship of seedling height to the absolute values of transfer distance for climate variables other than the annual moisture index were weak, with an $r^2 = 0.50$ ($p = 0.1839$) for the summer moisture index and with $0.4 < r^2 < 0.5$ for the remaining variables. In all cases, the sign of the relationship was negative, indicating that seedling height decreased as the difference between the climate of the test site and that of the provenance increased.

These results suggest that genetic differences among the populations have developed from selection along gradients of temperature–precipitation interactions. To be effective in limiting maladaptation in reforestation, seed zones should be based on genetic responses to the moisture index. Nonetheless, practical guidelines must relate the limits of seed transfer to altitude (Fig. 4) because maps of the moisture index do not exist. To this end, limits to seed transfer were defined from the confidence interval surrounding a predicted mean. This confidence interval can be used to define the geographic or climatic interval that must separate population before one can be reasonable certain of genetic differentiation. Although the confidence interval is dependent on experimental procedures
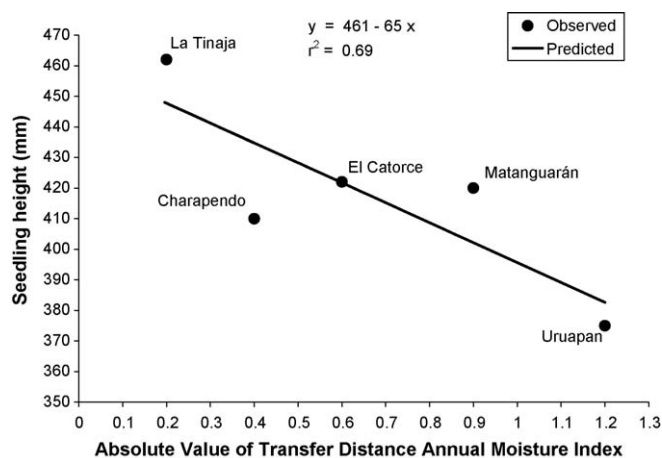

Fig. 3. Average 2.5-year-old *Pinus oocarpa* seedling height observed and predicted (by a linear model) against absolute value of transfer distance in units of average annual moisture index.
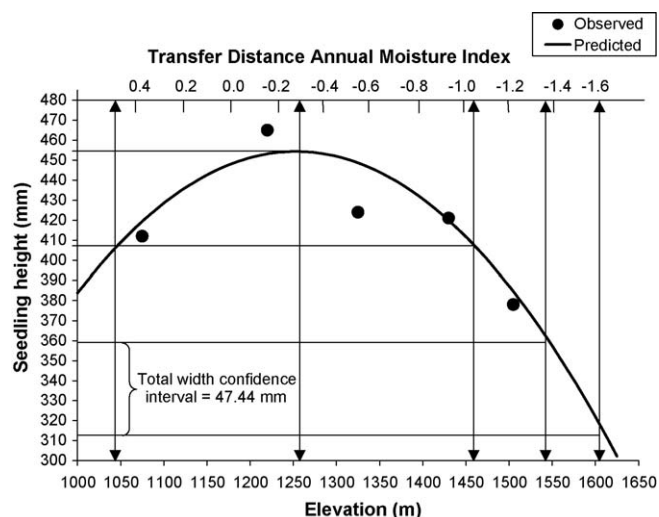
Fig. 4. Limits of four *P. oocarpa* seed zones, based on altitude and on transfer distance in annual moisture index units. Horizontal lines denote upper and lower 95% confidence interval limits for average seedling height. Vertical lines denote upper and lower limits for a seed zone in terms of altitude or transfer distance values in units of annual moisture index. Negative values of transfer distance denote transfers into a warmer and dryer climate than the provenance.

(such as sample size), using error structure for practical decisions is well established in applied quantitative genetics.

For seedling height at Canalejas, the confidence interval ($\alpha = 0.2$) was ± 23.72 mm, a value suggesting that seed zones should include populations differing by no more than 47.44 mm of average seedling height. With regard to the annual moisture index, seed zone breadth is readily calculated from the regression statistics: the ratio of the confidence interval to the regression coefficient (47.44/65) suggests that the breadth of a seed zone should be no more than 0.73 absolute units on the moisture index scale. Quite similarly, altitudinal limits could be defined from the regression of population elevation on seedling height (Fig. 4). For the example in Fig. 4, seed zones thus delimited in intervals starting from the maximum predicted height of 454 mm. By using both of these limits on expected responses of *P. oocarpa* populations, we delimited four seed zones that are consistent with both of sets of restrictions (Fig. 4 and Table 4).

As shown in Fig. 4, the breadth of a seed zones in relation to either altitude or to the moisture index is dependent on which part of the curve the zone is situated. Seed zones on the upper

altitudinal portion of the species distribution would be the narrowest (e.g. 66 m of altitudinal and 0.24 units of annual moisture index for seed zone 4) but would become broader where slope of the curve is less step (210 m of altitudinal range and 0.77 units of annual moisture index for seed zone 1, Table 4). Obviously, Fig. 4 illustrates one system of converting continuous variation into seed zones out of an innumerable array. Managers undoubtedly will adjust the zones to best represent their geographic region.

Average annual temperature, precipitation, degree days ($>5\,^{\circ}$C) and moisture index for the sampled populations and for the test site are presented in Table 3 for the contemporary climate and for climate projected for future decades. While contemporary temperatures range from 20.5 $^{\circ}$C at Uruapan population (1505 m) to 23.5 $^{\circ}$C at Charapendo population (1075 m), under the IS92a global warming scenario, temperatures should range from 24.3 to 27.0 $^{\circ}$C by year 2090, an average increase of 3.8 $^{\circ}$C. Precipitation at present ranged from 1640 mm at Uruapan to 1164 mm at Charapendo. However, precipitation is estimated to increase by year 2030, decrease by year 2060 and increase again by year 2090, eventually reaching a level of somewhat less than that of today. At present, degree days $>5\,^{\circ}$C range from 5643 at Uruapan to 6613 at Charapendo. By the end of the century, however, degree days are expected to rise to 7041 at Uruapan and to 8014 at Charapendo. This represents an average increase of approximately 23%.

The combined effect of the projected increase in temperature and slight decrease in precipitation would greatly increase the aridity of the climate. The moisture index currently ranges from 3.4 at Uruapan to 5.0 at Charapendo, but by year 2090, moisture indices should reach 4.4 at Uruapan and 6.2 at Charapendo, an average increase of 26% (larger moisture index values means more aridity). Although highest values of the annual moisture index are expected in 2090, the greatest change in the index is expected between 2030 and 2060. This result from a large decrease in precipitation projected between 2030 and 2060.

Table 4
Limits and ranges of four *Pinus oocarpa* seed zones delimited under two criteria: altitude and climatic transfer distance in units of annual moisture index (AMI)

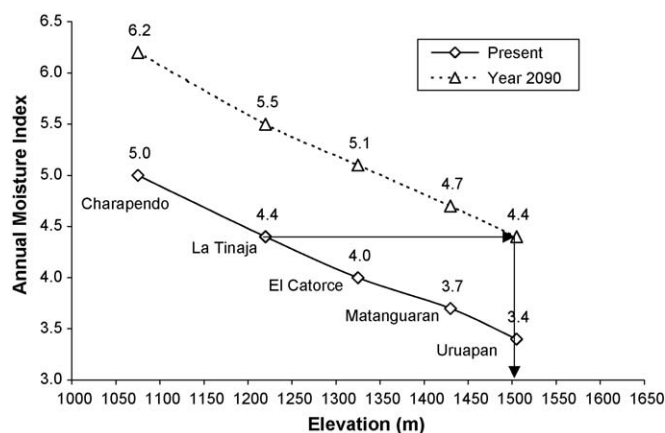| Seed zone number	Altitudinal criteria (m)			Climatic transfer distance criteria (AMI)		
	Limits			Limits		
	Lower	Upper	Range	Lower	Upper	Range
1	1045	1255	210	0.48	−0.29	0.77
2	1255	1461	206	−0.29	−1.05	0.76
3	1461	1547	86	−1.05	−1.37	0.32
4	1547	1613	66	−1.37	−1.61	0.24

Fig. 5. Estimated present and future (year 2090) annual moisture index for the studied region. Arrows indicate the corresponding altitude at which present annual moisture index of the La Tinaja population should occur by year 2090.

These changes in climate mean that the climate currently inhabited by a *P. oocarpa* population should occur at a higher elevation by year 2090 than it does today. For example, the La Tinaja population (1220 m) currently inhabits a climate with an average moisture index of 4.4, but by the end of this century, this same moisture index should typify the climate now inhabited by the Uruapan population at 1505 m. This suggests that the climate variable that seems to control adaptive differentiation of *P. oocarpa* populations is predicted to move upwards by 285 m in response to global warming (Table 3 and Fig. 5).

4. Discussion

4.1. Altitudinal genetic differentiation among populations

Genetic differentiation among populations tends to follow an altitudinal cline along which populations from lower altitudes generally have larger growth potential than populations from higher altitudes. This pattern likely results from differential selection pressures along the altitudinal gradient: populations from lower altitudes tend to be adapted to the milder climates under which selection has favored high growth potential, while populations from high altitudes display a lesser growth potential and greater tolerance of the cold (Rehfeldt, 1989, 1993, 1994).

However, in *P. oocarpa* this clinal pattern apparently is limited at the lowest altitudes by an increasing aridity of the climate. The population from the lowest altitude (Charapendo, 1075 m), has lower growth potential than three of the populations originating at higher altitudes (Fig. 3). It is quite likely that populations from the lowest altitudes have a more conservative growth strategy that allows drought stress to be avoided. This conclusion is consistent with the fact that Charapendo has a high annual moisture index (which means the highest aridity) and is located in the transition from montane forest to tropical dry forest: between La Tinaja and Charapendo, vegetation gradually changes from an increasingly dry pine–oak forest to a deciduous tropical dry forest dominated by legume trees and other species such as *Bursera* spp. In the transition zone, *P. oocarpa* becomes more and more rare such that locating the *P. oocarpa* population at Charapendo for this study was difficult.

For *P. oocarpa*, therefore, a high growth potential may indeed be characteristic of populations from the lower altitudes, but this does not necessarily mean that the highest growth potential occurs at the lowest extreme of the species' distribution. A similar patterning of variation was found for *Pinus brutia*, where provenances from the middle elevation zone (400–900 m) had greater growth than provenances from the periphery of the species' distribution (Isik et al., 2000). Similar trends have also been detected in *P. banksiana* (Mátyás and Yeatman, 1992) and *P. sylvestris* (Rehfeldt et al., 2003).

The results are consistent with the view that there is an optimal climate for the growth of each population (see also Mátyás and Yeatman, 1992; Rehfeldt et al., 1999b, 2003, 2004), and deviation in either direction from the optimum result in a loss of growth (e.g. when provenances from warm and dry climates are moved to more moist and colder climates – toward lower values of the moisture index, Fig. 2, or when provenances from cold and moist climates moved to warmer and dryer climates – toward higher values of the moisture index, Fig. 2). Although verification of these predictions could be made by testing of an array of provenances on multiple sites, the results are intuitive. Thus, the superior growth of La Tinaja population would reflect the fact that this population inhabits a provenance with a climate most similar to the climate of the test site; La Tinaja indeed has the lowest absolute value of transfer distance in units of annual moisture index, Table 3 and Fig. 3.

From a population genetics perspective, climatic differences along the altitudinal gradient apparently represent a selective pressure strong enough to partially override the effects of gene flow among populations. A previous study of isoenzymatic variation for the same *P. oocarpa* populations, indicated that there is an intense gene flow among these populations, and the five populations can be considered as a single panmitic population (F_{st} was not significantly different from zero, Sáenz-Romero and Tapia-Olivares, 2003). Despite the intense gene flow and the proximal location of the populations sampled (contiguous populations at 100 m of altitudinal difference and at 4 km of average geographic distance), significant differences exist among populations for growth traits. Apparently the swamping action of rampant gene flow has been compensated by differential climatic selective forces along the altitudinal gradient. Consequently, the selectively neutral isoenzymes are not capable of expressing the genetic differentiation among Uruapan region *P. oocarpa* populations (Sáenz-Romero and Tapia-Olivares, 2003) that can be detected by quantitative analyses.

While genetic variability among families within populations of most conifers is abundant, none was detected by our analyses. This lack of significant genetic differences among families could be due to either the relatively small differences among family averages, the small number of families within populations (six in average), or large variation within families (error term amounts to 88.9% of total variance, Table 2). It is relevant to note that in the nursery stage of this study, we found significant differences among families, but these early analyses included a larger number of families (approximately 11 families for each of the 5 *P. oocarpa* provenances). In addition, Mendizábal-Hernández et al. (1999) also found significant differences among 1-year-old half-sib families of *P. oocarpa*. It is likely, therefore, that family differences are real but were not detected in our analyses.

The best fitting regression model for explaining seedling height of populations was the quadratic regression driven by transfer distance in units of annual moisture index. Probably this is due to the fact that annual moisture index contains and combines information of the primary forces driving the natural distribution of the populations: temperature, precipitation, and their interaction. Seed transfer in reforestation, therefore, should be consistent with genetic clines along gradients of the moisture index. To be sure, elevation can be a strong surrogate for climate, but as shown in the present study, locations at

similar altitudes can have much different climates: e.g. Uruapan provenance (1505 m) is colder and more moist than Canalejas test site (1490 m). It is also possible for two sites with similar climate to have much different elevations: e.g. La Tinaja (1220 m) has at present an annual moisture index value (4.4) very similar to Canalejas test site (4.6), which is located at a much higher elevation. These observations, moreover, are consistent with the distribution of the native vegetation associations. The same transition from a pine–oak forest to a dryer tropical deciduous dry forest that occurs between La Tinaja and Charapendo (1220–1075 m) is repeated at a much higher elevation near to but slightly lower than the Canalejas test site.

4.2. Practical applications

The results have direct application in natural resource management, first for providing the scientific basis for delineating the seed zones used in artificial reforestation; second in dealing with issues surrounding ecological restoration; third, in devising programs in conservation of forest genetic resources; fourth, in developing tree improvement programs; fifth, for mitigating the effects of global warming.

4.2.1. Seed zoning

Seed zones not only must be scientifically sound but also must be practical to managers. In the present case, zones 3 and 4 undoubtedly are too narrow to be useful for management purposes (86 and 66 m of altitudinal range, respectively—Table 4). Consequently, we suggest merging the two seed zones at the highest altitude (seed zones 3 and 4 in Table 4) into a single zone which is called zone 3 in Table 5. Doing this still does not violate zonal limitations imposed by the annual moisture index (Fig. 4). As a result, each seed zone would have a breadth of 200 m of altitudinal and 0.75 units of the moisture index. Alternatively, for seed transfer guidelines, one can limit transfer of seeds from their source to either ±100 m of altitude or to ±0.375 units of the annual moisture index.

4.2.2. Ecological restoration

The primary objective in the reforestation of disturbed sites is to restore the coverage of the original forest (Sáenz-Romero

Table 5
Simplified limits and ranges of three *Pinus oocarpa* seed zones delimited under two criteria: altitude and climatic transfer distance in units of annual moisture index (AMI)

Seed zone number	Altitudinal criteria (m)			Climatic transfer distance criteria (AMI)		
	Limits			Limits		
	Lower	Upper	Range	Lower	Upper	Range
1	1050	1250	200	0.50	−0.25	0.75
2	1250	1450	200	−0.25	−1.00	0.75
3	1450	1650	200	−1.00	−1.75	0.75

Limits were rounded and the two highest altitudinal zones from Table 4 were merged in only one seed zone (named seed zone 3).

et al., 2003). This requires the use of genotypes adapted (i.e., physiologically attuned) to the climate at the sites to be reforested. For this objective, our results suggest, first, planting seedlings grown from seeds collected in the same seed zone (either within 200 m altitudinal zone or within 0.75 units of annual moisture index) where the site to be restored is located. If seed zones have not been delineated for the forested region as a whole, then seeds should be used for reforestation of only those sites within ±100 m of altitude of the collection site or within ±0.375 of units of annual moisture index of the collection site.

4.2.3. Genetic conservation

The high deforestation rate ongoing in Michoacán, particularly that associated with the replacement of natural stands of *P. oocarpa* by avocado orchards, demands urgent action for conservation of the remaining forest genetic resources. A first step is to establish Forest Genetic Resources Conservation Units (Sáenz-Romero and Tapia-Olivares, 2003). A Forest Genetic Resources Conservation Unit is a representative natural stand of any species with a management priority to maintain genetic diversity as well as to allow natural evolutionary forces to mold the population's genetic structure (Ledig, 1988, 1992; Millar and Libby, 1991; Sáenz-Romero et al., 2003). As shown in this study, altitudinal gradients parallel the pattern of genetic differentiation among populations. This means that one Forest Genetic Resources Conservation Unit established in each seed zone (Fig. 4) should capture a representative sample of the genetic diversity of *P. oocarpa* in the Uruapan region. The Forest Genetic Resources Conservation Unit also can be used as seed sources for restoring the original genetic structure of natural populations on disturbed or denuded lands. Guidelines for using seed originated from a Forest Genetic Resources Conservation Unit would be basically the same as for ecological restoration.

4.2.4. Tree breeding

The provenance with the highest growth potential, "La Tinaja" (1220 m of altitude), was 23% superior in seedling height than the provenance with the worst growth (Uruapan, located at 1505 m). However, such superiority cannot be considered as "realized genetic gain" as a result of moving upwards the provenance (from La Tinaja at 1220 m to Canalejas test site at 1490 m), because, as explained above, climate at the test site (Canalejas) actually is similar to climate at La Tinaja provenance. Thus, performance of La Tinaja provenance can be viewed as the performance of a provenance physiologically attuned to the local climate of the test site. A recommendation typical in tree breeding programs would be to use seeds from La Tinaja at Canalejas; otherwise, seedling growth would be inferior. In fact, the general recommendation supported by the quadratic regression model would be to use for sites like Canalejas seeds from about 1255 ± 100 m of the altitudinal range associated with the apex of the curve in Fig. 4. This altitudinal range would correspond to a site between populations La Tinaja and El Catorce (1325 m). However, to

transfer seeds from low elevations at La Tinaja to higher elevations at Canalejas would violate altitudinal limits of transfer of Fig. 4. This means, therefore, that long-distant transport of foreign provenances should only be done according to the moisture index. Yet, maps of moisture indices are not currently available in forestry; their development therefore should be a priority for those working on the improvement of *P. oocarpa*.

4.2.5. Mitigating effects of global warming

Increases in mean temperature and aridity (i.e. the annual moisture index) as a result of global warming, would create an adaptational lag (*sensu* Mátyás and Yeatman, 1992) in that the climate for which a population is best suited would have shifted to a novel location. For example, the climate for which the fastest-growing La Tinaja population is currently physiologically attuned (annual moisture index = 4.4), should occur by year 2090 at 1505 m, 285 m higher than at present (Fig. 5), while the present location of this population is expected to have a moisture index of 5.5, a value that exceeds the contemporary lower altitudinal limits of the species' distribution. The most difficult period for *P. oocarpa* populations in terms of adaptation to the new climatic conditions of the sites where they are growing, will occur between 2030 and 2060, when the increase in the annual moisture index should abruptly increase. This results because the temperatures are projected to increase while precipitation decreases, thus creating a period of a severe increment of arid conditions.

Unless managers actively promote a relocation of genotypes, contemporary populations will gradually become more and more poorly adapted as global warming progresses. Because of the speed projected for global warming, it seems unlikely that populations could migrate upwards in altitudinal at a rate necessary for maintaining adaptedness (Rehfeldt et al., 2004; Rice and Emery, 2003). For the lowest elevations, increased maladaptation eventually leads to extirpation (Rehfeldt et al., 2004), which appears to be the destiny of the *P. oocarpa* populations currently inhabiting the lowest elevations (Fig. 5).

Therefore, to assure adaptedness in the future, managers must soon begin assisting natural processes by moving genotypes upwards in their planting programs. However, the climate has not yet shifted sufficiently to warrant the transfer of seeds the full 285 m of altitude that is anticipated as necessary for assuring the adaptedness of La Tinaja population (1220 m) in 2090. A more appropriate approach for the present would be to move genotypes upwards a distance of less than one-half of the total altitudinal difference needed to match the climate anticipated for 2090. This would mean displacing contemporary populations an altitudinal distance between 100 and 150 m. This measure for the short term would achieve genetic gains (given that – in general terms – populations from lower altitudes have higher growth potential) and will assure adaptedness in the long term.

Acknowledgements

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